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Development and validation of NIR-Chemometric Method for Direct Determination of Gel Strength of Gelatin

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PURPOSE

Gelatin is a purified protein obtained from collagen of skin (Fig 1), bones, and connective tissues of animals such as cattle, pork and fish. Gelatin is obtained by partial hydrolysis (acid or basic) of collagen. One of the most important properties of gelatin is Gel Strength or Bloom strength. The Gel strength is the rigidity of gelatin gel and it is defined as the weight in grams that is required for a 12.7 mm diameter flat bottomed cylindrical plunger to depress the surface of a 6.67% (w/w) gelatin gel (matured at 10°C for 16-18 h) to a depth of 4 mm. The Gel strength analysis is performed using a texture analysis (Fig. 2) and between the sample preparation and analysis of Gel Strength almost 20 hours were spent. The United Standard Pharmacopoeia (USP) implemented this parameter and this method as indispensable in the process of quality control for the release of gelling grades gelatin, which delays the release process of the gelatin and therefore its utilization in the manufacturing process. For this reason, this paper shows the development of a fast, flexible and versatile method to analyze the Gel Strength. The method used Near Infrared (NIR) Spectroscopy and a chemometric technique called Partial Least Squares (PLS) Regression. The method will be able to determine the Gel Strength of gelatin, providing similar results of the USP method and reducing the time to do the analysis from 24 hours to 5 minutes.

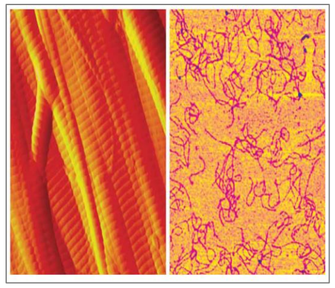

Fig. 1. Colagene [1]

Fig. 2. Texture Analizer

METHOD

NIR spectroscopy is an efficient and non-destructive method for the detection and quantification of physical and chemical characteristics[2]. Given that NIR analysis provides a large amount of data, it requires of chemometric methods, such as PLS, in order to perform the quantitative analysis. A PLS method involves the following steps: data collection, data preprocessing, building the calibration model, optimization and validation of the model[3]. Finally, the model can be used to predict gelatin samples where the Gel Strength is unknown. For this research, 33 samples were collected (Fig. 3), 25 samples were used for building the model and 8 samples were used to validate it . These samples were obtained from different manufacturers, sources (bone, skin cattle and skin Pork), types (acid and basic hydrolysis) and Gel Strengths (100 to 300g). All these gelatin samples were analyzed by the USP reference method and by NIRs spectra using diffuse reflectance.

Fig. 3. Gelatin Samples

Fig. 4. NIRFlex® N-500 by BUCHI

NIRs spectra were collected using NIRFlex® N-500 by BUCHI (Fig. 4) in a range of 800 to 2500 nm (Fig. 5). Then several pretreatments were used to improve the data and build the PLS calibration model. Four PLS models were obtained and evaluated using crossvalidation, left one out and validated using an external dataset.

To choose the best PLS model, different figures of merits were evaluated. The selected model was the PLS model with the lowest values in the figures of merits in the calibration model (Standard Error of Calibration (SEC), correlation coefficient (R), determination coefficient (R2)) and in the crossvalidation evaluation (Standard Error of Cross Validation (SECV), R and R2. Finally, the model was validated using an external data set and evaluating the following figures of merits: Standard Error of Prediction (SEP), Root Mean Square Error of Prediction (RMSEP), systematic difference or BIAS and percent of recovery (%Rec)[3].

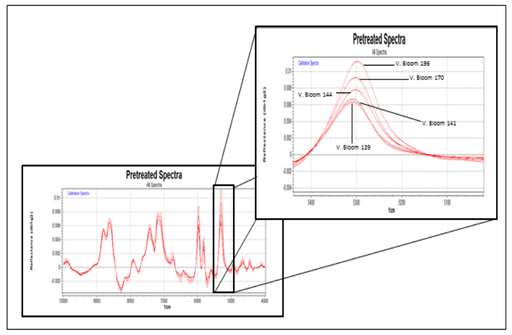

Fig. 5. NIR Spectra of Gelatin Samples and main zone of the spectra. In this zone the abortion band increase while the Bloom value increase

RESULTS

Once the four PLS models were evaluated, the model number 2 was selected as the best one with predictive ability (Fig 6). This model uses the whole NIR spectra and without pretreatment and using 10 PLS factors. This model showed the lowest results for calibration, crossvalidation and validation sets. The results obtained for this model were: Calibration Model: SEC= **0.6187**; R=**0.9995**; R^2=**0.9990**. Crossvalidation: SECV=**0.5152**; R=**0.9995**; R^2=**0.9989**. Validation: SEP=**0.8404**; RMSEP=**0.7039**; BIAS=**0.1570**; %Rec=**101.56**; R=**0.9974** and R^2=**0.9949**.

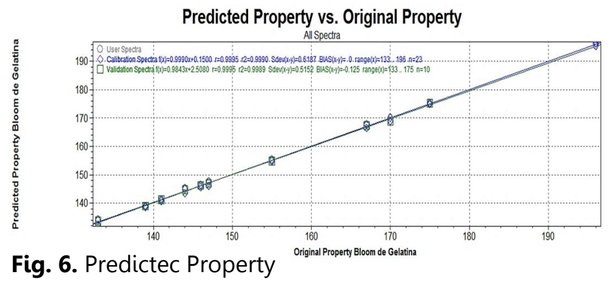

Fig. 6. Predictec Property

CONCLUSIONS

The results obtained show that the model selected is able to be used in order to determine the gel strength in the gelatin samples with high predictive ability, being an ideal model to predict the Gel Strength in gelatin samples.

The method developed to determine the Gel Strength in gelatin raw material by NIR Spectroscopy showed great potential for replacing the reference method (USP) in the laboratory, for its high predictive capacity with low prediction errors and acceptable recovery percent. With the implementation of this method, it is possible to obtain a Bloom Value for gelatin samples in periods of about 10 minutes compared to about 20 hours with the USP reference method.

REFERENCES

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